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Erysiphe javanica sp. nov., a new tropical powdery mildew from Indonesia

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Abstract — During the collection of tropical powdery mildews in Cibodas Botanical Garden and Mount Tangkuban Perahu in Indonesia, we found interesting specimens on *Castanopsis javanica* (*Fagaceae*). Phylogenetic analysis using a combination of 28S and ITS rDNA sequences clearly showed that this fungus forms a distinct lineage among the *Erysiphe* species found on hosts of the plant genus *Castanopsis*. Therefore, we consider this fungus a new species of *Erysiphe*, described here as *Erysiphe javanica*. Differences between *E. javanica* and closely related *Erysiphe* species are discussed.

Key words — Asia, *Brasiliomyces*, *Erysiphaceae*, phytopathogenic fungi, tropic

Introduction

Species of *Erysiphe* R. Hedw. ex DC. on fagaceous plants have been reported as a unique group of powdery mildews because they comprise not only fungi with simple mycelioid appendages that are characteristic for *Erysiphe* sect. *Erysiphe* (Braun & Takamatsu 2000), but also encompass taxa with different types of chasmothecial appendages previously belonging to the genera *Brasiliomyces* Viégas [rudimentary/mycelioid], *Uncinula* Lév. [uncinuloid], and *Microsphaera* Lév. [dichotomously branched at the apex] (Braun & Takamatsu 2000, Takamatsu et al. 2007, Divarangkoon et al. 2011). In addition, Divarangkoon et al. (2011) reported that the lineage of fagaceous *Erysiphe* spp. consists of not only taxa with multiple peridial cell layers but also species characterised by a single peridial cell layer previously assigned to *Brasiliomyces* [i.e., *E. trina* Harkn. (≡ *Brasiliomyces trinus* (Harkn.) R.Y. Zheng), *E. asiatica* Meeboon et al., and *E. monoperidiata* Meeboon et al. (Divarangkoon et al. 2011)]. These results suggest that different appendage types and single-layered peridial cells evolved on fagaceous hosts.

Several authors (Zheng 1984, Braun 1987, To-anun et al. 2003) have discussed the significance of the peridial cells in separating *Brasiliomyces* from *Erysiphe*. Zheng (1984) noted that *Brasiliomyces* must be treated as an independent genus due to having a single layer of peridial cells not differentiated into inner and outer layers as described in the genus *Cystotheca* Berk. & M.A. Curtis. This argument was supported by Braun (1987) based on the reexamination of the type species, *B. malvastri* Viégas. Before 2011, eight *Brasiliomyces* species had been described based on this morphological criterion (To-anun et al. 2003; Divarangkoon et al. 2011), of which there were recorded on fagaceous host plants — *B. trinus* (≡ *E. trina*), *B. cyclobalanopsidis* K.C. Kuo et al. (≡ *E. cyclobalanopsidis* (K.C. Kuo et al.) U. Braun), *B. kumaonensis* N. Ahmad et al. (≡ *E. kumaonensis* (N. Ahmad et al.) U. Braun). Braun & Paul (2009) placed these three species on fagaceous hosts in synonymy under *Erysiphe*, because *E. trina* nested in the lineage of *Erysiphe* emend. Braun & Takamatsu (2000).

During a March 2011 visit to the Cibodas Botanical Garden and Mount Tangkuban Perahu on Java Island (Indonesia), we found specimens morphologically similar to *E. asiatica* and *E. monoperidiata*. Molecular analyses showed that the rDNA sequences of the specimens form an independent lineage separated from *E. asiatica* and *E. monoperidiata* sequences. We therefore propose this fungus as a new species.

Materials & methods

Morphological examination

Specimens were collected in two locations of West Java province (Indonesia), namely, Cibodas Botanical Garden, Bogor and Mount Tangkuban Perahu, Bandung. All collections were conducted in March 2011. Details of host name, collection date, place, and collector were recorded. Morphological examinations were carried out as outlined in Divarangkoon et al. (2011). Mycelia and chasmothecia were stripped from leaf surfaces with a clean needle, mounted on a microscope slide, and examined in 3% NaOH using a light microscope with phase contrast 10×, 20×, and 40× objectives. Thirty chasmothecia, asci, and ascospores were measured per sample. Specimens were deposited at the National Museum of Nature and Science (TNS), Japan; Mie University Mycological Herbarium (MUMH), Japan; and Herbarium Bogoriense (BO), Indonesia.

Phylogenetic analysis

Whole-cell DNA was extracted from chasmothecia using the chelex method (Walsh et al. 1991) as described in Hirata & Takamatsu (1996). The 5′-end of the 28S rDNA, including the domains D1 and D2, and ITS region, including the 5.8S rDNA, were amplified by polymerase chain reaction (PCR) and then sequenced using protocols as described in Takamatsu et al. (2006). Representative sequences determined in this study were deposited in DNA databases (DDBJ, EMBL, GenBank) under the accession numbers of JQ220151–JQ220162. Sequences generated from the rDNA ITS region and D1/D2 domains were aligned using MEGA 5 (Kumar et al. 2008) with *Erysiphe*

and *Brasiliomyces* sequences retrieved from DNA databases (DDBJ, EMBL, GenBank). The alignment was deposited in TreeBASE (http://www.treebase.org/) under the accession number of S12121. Maximum parsimony (MP) analysis was done in PAUP* 4.0b10 (Swofford 2002) with the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting tree was tested with bootstrap analysis using 1000 replications (Felsenstein 1985). Tree scores, including tree length, CI, RI, and RC, were also calculated.

Taxonomy

Erysiphe javanica Meeboon & S. Takam., **sp. nov.** Fig. 1

MycoBank MB 563656

Similar to *Erysiphe asiatica*, but differing in 5–7-spored asci and occurrence on *Castanopsis javanica*.

Type: on *Castanopsis javanica* (Blume) A. DC. (*Fagaceae*), Indonesia, West Java province, Bogor, Cibodas Botanical Garden, 14 March 2011, J. Meeboon, I. Hidayat & S. Takamatsu (**Holotype**: TNS-F-44236; **isotype**: MUMH 5153; ex-type rDNA sequences, JQ220160 (28S), JQ220162 (ITS)).

Etymology: the species epithet refers to the island where the specimens were collected.

Colonies hypophyllous, persistent, forming irregular white patches on the host surfaces. Hyphae hyaline, superficial, septate, branched, 3–6 µm wide. Appressoria well-developed, coral-like, single or occasionally opposite in pairs. Conidiophores and conidia unknown. Chasmothecia scattered to

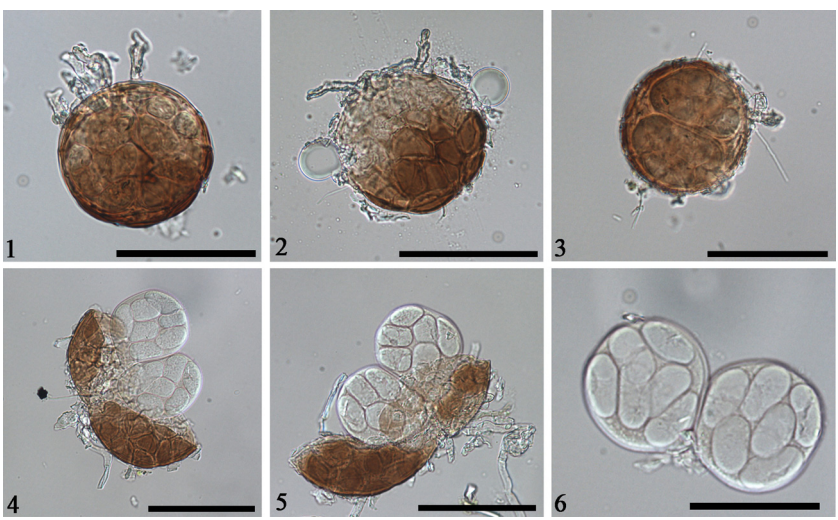

Fig. 1. *Erysiphe javanica* sp. nov.: 1–3, chasmothecia; 4–6, asci with 5–7 spores. Bars = 50 µm.

gregarious, (41–)50–66(–69) µm diam., globose, containing 2 asci; appendages present, rarely absent, poorly developed, mycelioid, (8–)17–73(–95) × (2–)3–5 (–8), colourless, aseptate, thin-walled, smooth. Peridium thin, one conspicuous layer, yellowish to light brown, semitransparent. Asci globose to subglobose, sessile or short-stalked, (30–)36–50(–76) × (26–)27–44(–53) µm, 5–7-spored. Ascospores ellipsoid-ovoid, hyaline, (10–)11–24(–26) × (5–)8–14(–17) µm.

Ecology & distribution: on *Castanopsis javanica,* Indonesia.

Additional collections examined: on *Castanopsis javanica:* **INDONESIA**. **West Java province**, Bogor, Cibodas Botanical Garden, 14 March 2011, J. Meeboon, I. Hidayat & S. Takamatsu (MUMH 5147, BO22660, BO22661, BO22662, BO22663); Bandung, Mount Tangkuban Perahu, 12 March 2011, J. Meeboon, I. Hidayat & S. Takamatsu (MUMH 5123, BO22655, BO22656, BO22657, BO22658, BO22659).

Phylogenetic analysis

In the 28S+ITS combination sequence data set, 1091 of 1277 total characters used in the MP analysis were constant, 58 characters were variable and parsimony-uninformative, and 128 characters were parsimony-informative. A total of 240 equally parsimonious trees (TL = 212, CI = 0.934, RI = 0.924, RC = 0.863) were generated by the MP analysis (Fig. 2). All *Erysiphe* species found on *Castanopsis* or *Lithocarpus* form a monophyletic clade with 98% bootstrap support. *Erysiphe javanica, E. asiatica,* and *E. monoperidiata* form independent clades with 99%, 100% and 66% bootstrap supports, respectively.

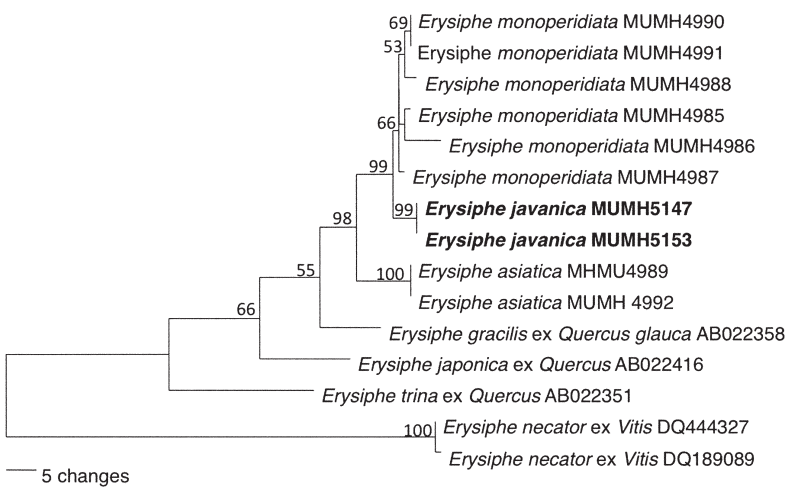

Fig. 2. Phylogeny of *Erysiphe javanica* inferred from a combination of 28S + ITS rDNA sequences using maximum parsimony (MP) method. Bootstrap support percentages (1000 replications; ≥50%) are shown on the branches.

Table 1. Morphological comparison of *Erysiphe javanica*, *E. monoperidiata*, and *E. asiatica*.

Character	*E. javanica*	*E. monoperidiata*	*E. asiatica*
Colonies	Hypophyllous	Amphigenous, mainly epiphyllous	Hypophyllous
Hyphae (µm diameter)	3–6	4–6	4–6
Chasmothecia (µm diameter)	(41–)50–66.5(–69.5) 2 asci	(55.5–)58–82.5(–85) 2–4 asci	(51–)57–74(–78) 2 asci
Appendage (µm)	(8.5–)17.5–73.5(–95) × (2–)3.5–5.5(–8)	(15.5–)18–66(–75) × (2.5–)3–6(–7.5)	(31–)45–51(–66) × (4–)4.5–5(–5.5)
Asci (µm)	(30.5–)36–50.5(–76) × (26–)27.5–44(–53) 5–7-spored	(34–)36–58(–61) × (24–)28–49(–52) 4–6-spored	(45–)46–59(–62) × (38–)40–53(–57.5) 6–8-spored
Ascospores (µm)	(10.5–)11.5–24.1(–26) × (5–)8–14(–17)	(11–)12.5–25(–26) × (6–)7.5–13(–14.5)	(16–)18–25(–28) × (8.5–)9–15(–16.5)

Discussion

Among the seven *Erysiphe* species previously found on *Castanopsis* (Braun 1987; Divarangkoon et al. 2011), only *E. asiatica* and *E. monoperidiata* were reported to have a chasmothecial peridium composed of a single cell layer (Divarangkoon et al. 2011). Morphologically, there are only slight morphological differences between *E. javanica, E. asiatica,* and *E. monoperidiata* (Table 1). *Erysiphe javanica* differs from *E. monoperidiata* in having hypophyllous colonies, smaller chasmothecia (69 µm diam. in *E. monoperidiata*), having only 2 asci (with 5–7 spores per ascus) per chasmothecium, and being found only on *C. javanica* in Indonesia. In addition, the morphologically close *E. asiatica* differs slightly in having 6–8 spored asci and being confined to *C. diversifolia* and *C. echinocarpa* in Thailand. Although it is difficult to differentiate *E. javanica* from *E. asiatica* and *E. monoperidiata* solely on morphology, our phylogenetic analysis, using a combination of 28S and ITS rDNA sequences, clearly showed that *E. javanica* forms a distinct lineage distinguishable from *E. asiatica* and *E. monoperidiata* (Fig. 2).

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